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Efficacy of 2-APB (2-aminoethyldiphenylborate) in Rescuing Neurons After Soman-Induced Brain Injury

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14. ABSTRACT

Soman produces seizures and seizure-related brain damage (SRBD). It is well known that termination of seizures using anticonvulsant drug therapy is the most effective means of preventing soman-induced SRBD. However, soman-induced seizures become refractive to anticonvulsant therapy within 40 minutes after their onset and the development of status epilepticus. Medical care for some battlefield casualties will likely be delayed beyond the therapeutic window of opportunity to terminate soman-induced seizures. Thus, there is a need for adjunct drug therapy that is neuroprotective when administered more than 40 minutes following soman exposure. Numerous evidence supports a pivotal role of sustained elevations in intracellular calcium (i.e., delayed calcium overload) in the development of brain damage resulting from seizures and status epilepticus. In addition, recent reports indicate that a sizable calcium influx occurs through transient receptor potential (TRP) channels, and this influx can be blocked by 2-aminoethyl diphenylborinate (2-APB; also called 2-aminoethoxy diphenylbroane and, misleadingly, 2-aminoethoxy diphenylborate). This study examined the possible neuroprotective effectiveness of 2-APB against soman-induced SRBD. Our results indicate that 2-APB (5.0 - 22.5 mg/kg in DMSO) was unable to ameliorate soman-induced SRBD. Moreover, we provide evidence that the DMSO vehicle (0.5 - 1.0 ml/kg) augmented temporal lobe lesions by soman.

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ABSTRACT

The nerve agent soman produces seizures and seizure-related brain damage (SRBD). It is well known that termination of seizures using anticonvulsant drug therapy is the most effective means of preventing soman-induced SRBD. However, soman-induced seizures become refractive to anticonvulsant therapy within 40 minutes after their onset and the development of status epilepticus. It is likely that medical care for some battlefield casualties will be delayed beyond the therapeutic window of opportunity to terminate soman-induced seizures. Thus, there is a need for adjunct drug therapy that is neuroprotective when administered more than 40 minutes following soman exposure. Numerous evidence supports a pivotal role of sustained elevations in intracellular calcium (i.e., delayed calcium overload) in the development of brain damage resulting from seizures and status epilepticus. In addition, recent reports indicate that a sizable calcium influx occurs through transient receptor potential (TRP) channels, and this influx can be blocked by 2-aminoethyl diphenylborinate (2-APB; also called 2-aminoethoxy diphenylbroane and, misleadingly, 2-aminoethoxy diphenylborate). The present study was undertaken to examine the possible neuroprotective effectiveness of 2-APB against SRBD resulting from soman exposure. Our results indicate that 2-APB (5.0 - 22.5 mg/kg in DMSO) was unable to ameliorate soman-induced SRBD. Moreover, we provide evidence that the DMSO vehicle itself (0.5 - 1.0 ml/kg) augmented temporal lobe lesions by soman.

INTRODUCTION

Protection against brain damage resulting from nerve agent exposure is of significant military concern. The current regimen of antidotal therapy most effectively addresses the acute life-threatening consequences of exposure. However, many soldiers surviving the initial life-threatening effects of nerve agent intoxication are likely to develop seizures. Anticonvulsants such as diazepam can arrest soman-induced seizures when administered shortly following seizure onset, but their effectiveness diminishes when treatment is delayed for 40 minutes or more (e.g., Lipp, 1972, 1973; Shih, 1990; Shih *et al*., 1991; Capacio and Shih, 1991; Philippens *et al*., 1992; Sparenborg *et al*., 1993; McDonough and Shih, 1993; Harris *et al*., 1994; Shih *et al*., 1999; Lallement *et al*., 2000; McDonough *et al*., 2000). Unless seizures are terminated, the currently fielded therapy does not afford complete protection against brain damage. Furthermore, it is very likely that there will be unconscious nerve agent exposure victims who suffer silent seizures. Since these seizures are not associated with the usual behavioral manifestations seen with typical seizures, victims may not receive any anticonvulsant treatment. If left untreated, or if they cannot be arrested, nerve agent-induced seizures progress to status epilepticus and lead to extensive brain damage. Therefore, there is a clear need for adjunct therapy that is capable of preventing brain damage even if seizures have progressed to status epilepticus and cannot be terminated. Such a compound would greatly increase the window of opportunity for the prevention of brain damage resulting from nerve agent-induced seizures and would augment the beneficial effects of currently fielded anticonvulsants.

Soman (O-1,2,2-trimethylpropylmethyl-phosphonofluoridate) is an organophosphorous nerve agent that produces status epilepticus and seizure-related brain damage (SRBD) (Petras, 1981; Lemercier *et al*., 1983; McLeod *et al*., 1984; McDonough *et al*., 1987). Seizure induction results from soman's ability to irreversibly inhibit acetylcholinesterase (AChE), causing an elevation in acetylcholine concentration in the brain (reviewed in Solberg and Belkin, 1997). Once initiated by elevated acetylcholine concentrations in susceptible brain regions, seizures are maintained by excess glutaminergic synaptic transmission (Olney *et al*., 1983; Sparenborg *et al*., 1992; Solberg and Belkin, 1997). It is well known that glutamate receptor abuse produces excitotoxicity and neuronal cell death. Moreover, the mechanism by which glutamate excitotoxicity causes neuronal death is dependent on sustained elevations in intracellular free calcium (i.e., delayed calcium overload) (Olney *et al*., 1983, 1987; Choi, 1987, 1988). There is considerable evidence that soman-induced seizure-related brain damage (SRBD) results from glutamate excitotoxicity and the ensuing delayed calcium overload. For example, it has been reported that SRBD resulting from soman-induced seizures can be alleviated by administration of various N-methyl-D-aspartate (NMDA) receptor antagonists: MK-801, GK-11, TCP and HU-211 (Olney *et al*., 1983; Braitman and Sparenborg, 1989; Shih, 1990; Sparenborg *et al*., 1992; McDonough and Shih, 1993; Lallement *et al*., 1993, 1994, 1997; Solberg and Belkin, 1997; Filbert *et al*., 1999; Carpentier *et al*., 2001a, 2001b, De Groot *et al*., 2001). The NMDA receptor is a ligand-gated calcium channel, and the above antagonists counteract delayed calcium overload by blocking calcium influx. Unfortunately, NMDA receptor antagonists have the drawback of being neurotoxic themselves. Neurotoxicity in the posterior cingulate and retrosplenial cortices has been reported following their use (Olney *et al*., 1989; Fix, 1994; Wozniak *et al*., 1998). Additional evidence indicates that a sizable portion of the intracellular free calcium that contributes to neuronal pathogenesis comes from intracellular stores (i.e., the endoplasmic

reticulum, ER) (Randal and Thayer, 1992; Mody and MacDonald, 1995; Yoon *et al*., 1996; Wei and Perry, 1996; Neibauer and Gruenthal, 1999; Pelletier *et al*., 1999; Yu *et al*., 1999; Nakayama *et al*., 2002; Verkhratsky and Toescu, 2003). The ryanodine receptor antagonist dantrolene has been shown to diminish soman-induced SRBD by blocking calcium release from the ER (Ballough and Filbert, 2003).

Very recent evidence points to the possible involvement of a new class of membrane ion channels in the mediation of calcium flux responsible for delayed calcium overload and neuronal cell death. Transient receptor potential (TRP) channels have been referred to as "the last bastion of ion channels" (Clapham *et al*., 2001). They are also considered the most likely ion channel candidates responsible for "capacitative calcium entry" (reviewed in Putney, 2003). Capacitative calcium entry is a process whereby the depletion of calcium from intracellular stores (i.e., ER) causes opening of calcium permeable channels in the plasma membrane. It has been proposed that these channels are activated following neurotransmitter receptor activation, by fast acting neurotransmitters, and facilitate rapid replenishment of intracellular stores. These channels remain open until intracellular stores of calcium are completely replenished and provide a means for inducing prolonged, sustained calcium elevations in intracellular calcium (Putney, 2001; Putney, 2003). Not only are TRP channels the most likely candidates responsible for sustained calcium elevations associated with capacitative entry, but their activation has also been linked to delayed calcium overload resulting from glutamate excitotoxicity and neuronal cell death (Aarts *et al*., 2003: Chinopoulos *et al*., 2004; Moran *et al*., 2004). Recent findings by Chinopoulos *et al*. (2004) indicate that delayed calcium entry in cortical neurons, excitotoxicity-induced, is diminished by the TRP channel antagonist 2-APB.

As a point of clarification, it should be noted that "2-APB" (or 2APB) correctly refers to 2-aminoethyl diphenylborinate or 2-aminoethoxy diphenylborane; however, it has been misleadingly called "2-aminoethoxy diphenylborate" in the vast majority of literature (Chawla *et al*., 2001). This TRP channel antagonist also inhibits IP3 ionotropic receptors, i.e., ligand-gated calcium channels that are responsible for second-messenger mediated release of calcium from the ER (Maruyama *et al*., 1997). Therefore, 2-APB has the potential of diminishing delayed calcium overload in two ways: (1) by blocking calcium influx through TRP channels and (2) by preventing calcium efflux from the ER following matabotropic glutamate receptor (mGluR) stimulated increase in the IP3 second messenger. The present study was undertaken to investigate the possibility that 2-APB administration, in conjunction with oxime and atropine treatments, may augment neuroprotection against brain damage resulting from soman-induced seizures and status epilepticus.

METHODS

Fifty-one male Sprague-Dawley rats (CRL: CD[SD]-BR; Charles River Labs, Wilmington, MA), weighing between 250-300 g, were used. Animals were housed individually in polycarbonate cages under conditions of constant temperature (21 ± 2°C) and humidity (50 ± 10%), using at least 10 complete air changes per hour of 100% fresh air, and a 12-hour light-dark cycle (full spectrum lighting cycle with no twilight). Throughout the study, food and water were available *ad libitum*, except during the observation period, which began 1.5 hours prior to and ended 6 hours following soman administration.

Representative rats from each group were anesthetized with sodium pentobarbital (50 mg/kg, diluted in saline to give an intraperitoneal [i.p.] injection volume of 3.3 ml/kg) and positioned in a stereotaxic apparatus (David Koff Instruments, Tujunga, CA); for most subjects, supplemental injections of dilute pentobarbital (i.e., 10 ± 3 mg/kg) were given prior to completion of surgeries, as needed. Prior to incisions, subcutaneous (s.c.) xylocaine was administered for local analgesia. Placement of screw electrodes was performed in accordance with the procedure recommended by Braitman and Sparenborg (1989) for electrocorticographic (ECoG) recordings. Electrodes were connected to a standard small-animal head-piece and secured by dental cement.

On the morning of the fifth or sixth day following surgeries, electrode-implanted animals were connected to an ECoG recording system and allowed at least 30 min to acclimate. Baseline ECoG activity and behavior were monitored for at least 15 min. Following baseline recordings, animals were injected (i.p.) with 125 mg/kg of the oxime HI-6. This was followed 30 min later by injection of 180 μg/kg soman (1.6 LD_{50}, s.c.) or sterile saline. Within one min following soman or saline injection, animals were injected intramuscularly (i.m.) with 2 mg/kg atropine methylnitrate (AMN). All rats, with the exception of those belonging to the untreated control group, received HI-6 and AMN. These were employed to protect against the peripheral effects of soman and to reduce mortality without affecting seizures. Seizure onset, following soman administration, corresponded to the initiation of sustained ECoG amplitudes greater than four times baseline (e.g., McDonough and Shih, 1993). For the remaining rats in each group that were not instrumented for ECoG recordings, seizures were inferred from overt manifestations (see below) including Straub tail; seizure onset was marked by the occurrence of the latter. Test drugs were administered 5 minutes following seizure onset (see below). At the end of the 6-h observation period, each soman-exposed rat received a supplementary injection of isotonic saline (5 ml, i.p.) to prevent dehydration.

Test drugs included 2-aminoethyl diphenylborinate (2-APB; Sigma-Aldrich Co.) and ultra pure dimethyl sulfoxide (DMSO; Sigma-Aldrich Co.) vehicle. Treatment groups were as follows:

1)	Soman-positive controls	n = 6
2)	Soman + 2-APB (22.5 mg/kg) in DMSO (1.0 ml/kg)	n = 3
3)	Soman + 2-APB (13.7 mg/kg) in DMSO (0.7 ml/kg)	n = 11
4)	Soman + 2-APB (10.0 mg/kg) in DMSO (1.0 ml/kg)	n = 3
5)	Soman + 2-APB (0.5 mg/kg) in DMSO (0.5 ml/kg)	n = 10
6)	Soman + DMSO (1.0 ml/kg)	n = 4
7)	Soman + DMSO (0.7 ml/kg)	n = 6
8)	Soman + DMSO (0.5 ml/kg)	n = 4
9)	Non-Soman + 2-APB (22.5 mg/kg) in DMSO (1.0 ml/kg)	n = 2
10)	Untreated controls	n = 2

Twenty-nine ± 1 hours after soman administration, rats were given a lethal injection of pentobarbital anesthesia (130 mg/kg, i.p.) and euthanized, upon evidence of labored breathing, via transcardial perfusion with ice cold 10% NBF. Brains were excised and post-fixed in 10% NBF for approximately 24 hours prior to tissue processing. Subsequently, brain specimens were paraffin processed and coronally sectioned at 4 μm using a rotary microtome. Two brain

sections (stereotaxic coordinates between bregma -2.64 and -3.48 [Paxinos and Watson, 2005]) from each animal were collected for hematoxylin and eosin (H&E) or microtubule associated protein 2 (MAP-2) histo- or immunohistochemical processing, respectively. H&E stained brain sections were assessed for classical histopathological damage to the piriform cortex. Damage was scored on a scale of 0 to 4, where 0 = no histologic lesion, 1 = minimal damage (1-10% neuronal loss), 2 = mild (11-25% neuronal loss), 3 = moderate (26-45% neuronal loss) and 4 = severe (> 45% neuronal loss). MAP-2 immunostained brain sections were ranked according to severity of temporal lobe necrosis and were further employed for image analysis assessments (see below).

MAP2 immunohistochemistry utilized a monoclonal antiserum, raised in mice, against microtubule-associated protein 2 (MAP2) (Sigma Chemical Co., St Louis, MO), and employed the avidin-biotin-peroxidase method of Hsu *et al*. (1981). Morphometric image analysis of MAP2 immunostained brain sections was performed using an image analysis system obtained through I-CUBE Inc. (Glen Burnie, MD). This system included Image-Pro Plus v4.1 software, Sony Power HAD camera, Hitachi CM771 monitor and I-CUBE computer. The system was interfaced with an Olympus BH-2 Biological Microscope (Olympus Optical Co., Ltd., Tokyo, Japan). Cross-sectional areas of MAP2 negative staining (i.e., necrosis [Ballough *et al*., 1995; Hicks *et al*., 1995]) in piriform cortex and contiguous brain regions (e.g., amygdaloid nuclei and perirhinal cortex) was performed according to the procedure of Ballough *et al*. (1995). Previous studies have shown that the piriform cortex (with surrounding brain regions) presents the most clearly defined and easily quantifiable lesions of contiguous necrosis following soman-induced seizures in rats (e.g., Ballough *et al*., 1995).

H&E and MAP2 qualitative damage ratings were grouped according to treatment and brain region, and compared using Mann-Whitney nonparametric statistical analyses. Cross-sectional areas of contiguous necrosis (MAP2 negative) were grouped by treatment and compared using one-way analysis of variance (ANOVA), followed by Student Newman Keul (SNK) multiple range test. Values for $p < 0.05$ were considered significant.

RESULTS

All soman-treated rats exhibited sustained seizures and status epilepticus for several hours. Proconvulsive behavioral signs of soman intoxication included repetitive chewing, facial and forepaw clonus, motor stereotypy, and wet-dog shakes. Overt motor convulsions were characterized by rhythmic clonic jerks of both head and forepaws, rearing, salivation and Straub tail. Non-soman control rats showed no evidence of seizures or convulsions. Visual inspection of ECoG recordings, from representative animals of each group, revealed no evidence of altered seizure activity by 2-APB or DMSO, compared with soman-positive controls.

Mortality appeared to vary with treatment group and seemed most affected by DMSO dosage. Of the 6 rats belonging to the soman-positive control group, 1 died prior to sacrifice. Of the 10 rats that received 1.0 ml/kg DMSO (with and without 2-APB), only 2 survived; survivors included 1 rat that also received 10 mg/kg 2-APB (i.e., 1 of 3 rats), and 1 rat in the 1.0 ml/kg DMSO soman-positive control group (i.e., 1 of 4 rats). All 3 rats died that received 22.5 mg/kg 2-APB in 1.0 ml/kg DMSO. Of the 17 rats that received 0.7 ml/kg DMSO (with and without 2-

APB), 12 survived; survivors included 7 of 9 rats that also received 13.7 mg/kg 2-APB, and 5 of 6 rats in the DMSO (0.7 mg/kg) soman-positive control group. Of the 14 rats that received 0.5 ml/kg DMSO (with or without 2-APB), 9 survived; survivors included 6 of 10 rats that also received 5 mg/kg 2-APB, and 3 of 4 rats in the DMSO (0.5 ml/kg) soman-positive control group.

Histopathological evaluations of MAP2 and H&E-stained brain sections from all surviving soman-exposed animals, irrespective of treatment, revealed severe region-specific brain damage. Damage was bilaterally symmetrical and characterized by widespread tissue necrosis, neuronal loss, chromatolysis, vacuolization, pyknosis and gliosis. Between bregma -2.64 and -3.48mm, severe damage was consistently observed in the piriform and entorhinal cortices, dorsal endopiriform nucleus and the laterodorsal thalamic nucleus. Pronounced damage was often seen in the perirhinal cortex, amygdaloid complex, hippocampus, midline thalamic nuclei, and ventrolateral thalamic nuclei. The pattern of soman-induced seizure-related brain damage (SRBD) seen in the present study is consistent with previous reports (e.g., Petras, 1981; Lemercier *et al*., 1983; Pazdernik *et al*., 1985; Carpentier *et al*., 1990; Ballough *et al*., 1995, 1998; McDonough *et al*., 1998). Non-soman, 2-APN/DMSO control rats showed no evidence of neuropathology.

Histopathological damage ratings for H&E-stained brain sections are based on the presence of necrotic neurons and/or the absence of a defined neuronal population. Shrunken neurons are considered the result of artifactual change. Damage to the neuropil is progressively greater as ratings increase from “mild” to “severe,” and is characterized by increasingly severe malacia and hyalinization typical of necrosis. Qualitative MAP2 damage ratings (1-10) were based exclusively on the presence and severity of temporal lobe necrosis, as delineated by MAP2-negative immunostaining. A rating of "1" indicates no necrosis and is the typical rating assigned non-soman control animals. A rating of "10" is assigned when necrosis is so widespread that the entire piriform cortex is necrotic, and necrosis extends dorsally and medially to include perirhinal cortex and amygdaloid nuclei, respectively. Group means for H&E and MAP2 damage ratings, as well as cross-sectional areas of necrosis are presented in Table 1.

Table 1

Treatment Group (Surviving Soman-Exposed Rats)	N	MAP2 Ratings (Mean ± SEM)	H&E Ratings (Mean ± SEM)	MAP2 Necrosis (mm^2) (Mean ± SEM)	(Mean ± SEM)
Soman-Pos Controls	5	7.00 ± 1.14	2.60 ± 0.68*	5.49 ± 1.63	
2-APB 13.7mg/kg (in DMSO 0.7)		7	8.71 ± 0.42	4.00 ± 0.00	5.69 ± 0.81
2-APB 5.0mg/kg (in DMSO 0.5)		6	7.50 ± 1.06	3.00 ± 0.45	5.41 ± 0.37
DMSO 1.0 ml/kg	1	10.0 ± N/A	4.00 ± N/A	8.56 ± N/A	
DMSO 0.7ml/kg	5	8.40 ± 0.68	4.00 ± 0.00	6.66 ± 0.85	
DMSO 0.5ml/kg	3	10.0 ± 0.00	4.00 ± 0.00	8.39 ± 1.59	
Combined 2APB	13	8.15 ± 0.54	3.54 ± 0.24	5.56 ± 0.45*	
Combined DMSO	9	9.11 ± 0.46	4.00 ± 0.00*	7.45 ± 0.71*	

Damage Assessments: Respective group means for MAP2 (1-10 ratings) and H&E (0-4 ratings) were compared using Mann-Whitney nonparametric statistical analyses. Mean cross-sectional areas of temporal lobe necrosis (MAP2 negative immunostaining) were compared using ANOVA followed by SNK. Significant differences ($p<0.05$) were observed between underlined means indicated by asterisks. Underlined means without asterisks (i.e., MAP2 ratings) showed a tendency for difference (i.e., $p=0.063$).

It can be seen that piriform cortical damage was not diminished by any dosage of 2-APB in the DMSO vehicle. Moreover, from visual inspection of mean damage ratings (Table 1), it would appear that damage was increased in groups receiving 2-APB compared with soman-positive controls. This, however, does not bear out statistically. No significant differences were found between the individual treatment groups receiving 2-APB (i.e., 13.7 and 5.0 mg/kg, in DMSO) and the soman-positive control group for any damage assessment method. In addition, no differences were found between the combined 2-APB group (i.e., pooled data from the 13.7 and 5.0 mg/kg 2APB groups) and soman-positive controls. If a statistical "type-2 error" has occurred here (i.e., incorrectly concluding no differences between means), it is likely the result of the uncharacteristically high variability within the present soman-positive control group (i.e., compared with soman control groups of previous studies; data not shown) and insufficient animal number in the soman control group.

Despite inconsistencies within the soman-positive control group, a significant difference was found between mean H&E damage ratings of the combined DMSO group and soman controls. Although these DMSO subjects were initially intended as vehicle controls, average damage H&E ratings were significantly higher in this group (4.00 ± 0.00) compared with soman controls (2.60 ± 0.68); $p = 0.043$ with Mann Whitney non-parametric analysis. Elevated pathology in the combined DMSO group compared with soman controls was somewhat corroborated by MAP2-negative (necrosis) ratings. Necrosis ratings were 9.11 ± 0.46 and 7.00 ± 1.14 in the combined DMSO and soman control groups, respectively; however, the difference between the groups was not significant (i.e., $p = 0.063$). Quantitative assessments of temporal lobe necrosis did not reveal a significant difference between soman controls (5.49 ± 1.63 mm^2) and the combined DMSO group (7.45 ± 0.71 mm^2), but did show a significant difference ($p = 0.029$ using ANOVA) between the latter group and the combined 2-APB (5.56 ± 0.45 mm^2). Thus, the combined DMSO vehicle control group exhibited a 34.0% increase in temporal lobe necrosis

(i.e., piriform cortex and contiguous areas) compared with the combined 2-APB in DMSO group.

DISCUSSION

The present findings do not demonstrate clear neuroprotective efficacy by 2-APB against SRBD resulting from soman-induced seizures. In Table 1, it can be seen that 5.0 and 13.7 mg/kg 2-APB (in 0.5 and 0.7 ml/kg DMSO, respectively) did not reduce cross-sectional areas of temporal lobe necrosis compared with soman-positive controls. In fact, H&E and MAP2 damage ratings appeared higher (not significantly) for these groups compared to soman controls. To increase statistical power, data for the above 2-APB groups were pooled into a combined 2-APB group and compared with soman controls. Still, no significant differences were seen between the combined 2-APB group and soman controls with any of the damage assessments. On the other hand, soman-exposed rats that received the DMSO vehicle showed exacerbated damage compared with soman controls, in two of three pathological assessments. A significant increase in mean H&E damage ratings was seen in the combined DMSO group compared with soman-positive controls (i.e., mean H&E ratings were 4.00 and 2.60, respectively, $p=0.043$). A tendency for increased damage was also seen in the combined DMSO group compared to soman controls based on MAP2 qualitative ratings (i.e., mean ratings were 9.11 and 7.00, respectively, $p=0.063$). Despite seemingly different means, a significant difference between these two groups was not obtained from image analysis data representing cross-sectional areas of temporal lobe necrosis (means were 7.45 and 5.49 mm^2 for the combined DMSO and soman controls, respectively). Interestingly, a significantly higher cross-sectional area of necrosis was seen in the combined DMSO group compared with the combined 2-APB group (means were 7.45 and 5.56 mm^2, respectively, $p=0.029$). That significance was obtained in the latter comparison, but not in the former, is likely explained by greater than normal variability within the soman-positive control group (compared to previous studies) and few subjects within the group (i.e., n=5 survivors).

The present findings, that DMSO contributed to brain pathology resulting from soman-induced seizures, are difficult to reconcile in light of its widespread use and well characterized effects in mammalian systems. Early toxicological studies indicate that rats can tolerate chronic administration of 9 ml/kg/day DMSO before showing any signs of toxicity (Noel *et al*., 1975). There have been numerous reports of free radical scavenging and other beneficial effects of DMSO, in rats, at dosages between 1.0 and 6.0 ml/kg (e.g., Wang *et al*., 2000; Lind *et al*., 2000; Chang *et al*., 2001; Nakamuta *et al*., 2001). Moreover, DMSO toxicity has not been observed in previous studies involving nerve agent administration (e.g., Bodjarian *et al*., 1995). In 1966, Loomis and Johnson reported a "reversal of soman-induced effects on neuromuscular function with oximes in the presence of dimethyl sulfoxide." On the other hand, it was recently reported that DMSO produces osmotically induced nerve structural changes, ion channel block, and membrane fluidity changes (Larsen *et al*., 1996). With respect to the osmotic effects of DMSO, it was reported in 1972, by De Bruijne and Van Steveninck, that DMSO can induce osmotic swelling and lysis in various cell types. Alternatively, Kubota *et al*. (1998) reported an enhancing effect of DMSO on neural transmission that was not explained by osmotic effects or blockade of potassium channels, but rather by inhibition of cholinesterase activity or other actions involved in increasing transmitter release from nerve endings. In the present study, it is

also possible that DMSO displaced soman that was absorbed into body fat, rendering proportionately more soman available to affect the central nervous system (personal communication from MAJ J.S. Estep, DVM DACVP). Irrespective of whether DMSO increased necrosis by (1) potentiating osmotic swelling and lysis in neurons already rendered vulnerable by metabolic compromise and ionic imbalances, (2) enhancing cholinesterase inhibition, (3) exacerbating excitotoxicity by facilitating neuronal firing, or (4) effectively increasing soman dosage by displacement from body fat, the present findings give reason for caution when using this vehicle in nerve agent models.

In light of the fact that a significant reduction in mean cross-sectional area of temporal lobe necrosis was seen in the combined 2-APB-treated group compared with the combined DMSO group, it is possible that TRP channel inhibition as well as IP3 receptor antagonism, by 2-APB, provided some measure of neuroprotection against the combined soman/DMSO insult. However, considering the magnitude of residual brain damage following 2-APB treatment, the slight neuroprotective efficacy it offers is deemed insufficient to warrant its further investigation in nerve agent models.

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